FEBS Letters 587 (2013) 3668–3674

FEBS Letters

journal homepage: www.FEBSLetters.org

Caspase-mediated cleavage and DNase activity of the translation initiation factor 3, subunit G (eIF3g)

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ARTICLE INFO

Article history:
Received 13 June 2013
Revised 12 September 2013
Accepted 20 September 2013
Available online 27 September 2013

Edited by Barry Halliwell

Keywords:
Translation initiation factor
eIF3g
Caspase
DNase
Apoptosis

ABSTRACT

Eukaryotic translation initiation factor 3 is composed of 13 subunits (eIF3a through eIF3m) and plays an essential role in translation. During apoptosis, several caspases rapidly down-regulate protein synthesis by cleaving eIF4G, -4B, -3j, and -2α. In this study, we found that the activation of caspases by cisplatin in T24 cells induces the cleavage of subunit G of the eIF3 complex (eIF3g). The cleavage site (SLRD^{220}G) was identified, and we found that the cleaved N-terminus was translocated to the nucleus, activating caspase-3, and that it also showed a strong DNase activity. These data demonstrate the important roles of eIF3g in the translation initiation machinery and in DNA degradation during apoptosis.

1. Introduction

Gene expression is regulated at both transcriptional and translational levels, and its deregulation results in abnormal gene expression or apoptosis. The efficiency of translation initiation determines the rate of protein synthesis involves complex interactions between many translation initiation factors (eIFs) and RNA molecules, and requires protein interactions between individual eIF subunits and ribosomes. Among the 11 eIFs, eukaryotic translation initiation factor 3 (eIF3) is the most complex factor and plays a central role in the initiation pathway [1–4]. eIF3 binds directly to the 40S ribosomal subunit and promotes the binding of Met-tRNA and mRNA. Additionally, eIF3 binds directly to eIF1, eIF4B, eIF4G, and eIF5 through its individual subunits. In mammalian cells, the eIF3 complex has a molecular weight of approximately 700 kDa and consists of 13 multi-subunits: eIF3a (p170), -b (p116), -c (p110), -d (p66), -e (p48), -f (p47), -g (p44), -h (p40), -i (p36), -j (p35), -k (p28), -l (p67), and -m (GA17) [5–7]. Among them, eIF3a (Tif32p in yeast *S accharomyces cerevisiae*), eIF3b (Nip1p), eIF3c (Prt1p), eIF3g (Tif35p), and eIF3i (Tif34p) comprise the eIF3 core complex [8] and are conserved in yeast and mammalian cells.

Apoptosis is generally associated with the collapse of the protein translation machinery, which is mediated by caspase activation [9]. When executioner caspases are activated by initiator caspases or other signals that do not depend on a caspase cascade, these enzymes cleave critical proteins that are required for the maintenance of cellular homeostasis, including the translation initiation machinery. The inhibition of translation is accompanied by the modification of translation factors, such as the phosphorylation of eIF2α and the caspase-mediated cleavage of initiation factors eIF4G [10–12], eIF4B [13], eIF4E-binding protein (eIF4E-BP1) [13], eIF2α [14], and eIF3j [13]. eIF4G is cleaved by caspases at a region between the eIF4E and eIF3 binding sites, causing the loss of the bridging function for the eIFs and mRNA circularization. eIF4B, eIF3j, and eIF2α are also cleaved by caspase-3 during serum starvation or tumor necrosis factor α (TNF-α) treatment.

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0014-5793/$36.00 © 2013 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.febslet.2013.09.027

We previously reported that apoptosis-inducing factor (AIF), which is capable of translocating from mitochondria to the nucleus during apoptosis, inhibits protein synthesis by interacting with eIF3g [15]. In this study, we demonstrate that eIF3g is cleaved by caspases-3 and -7 during apoptosis and that cleaved-eIF3g demonstrates DNase activity.

2. Materials and methods

2.1. Cell culture and reagents

T24 bladder carcinoma cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Staurosporine (STS; Calbiochem, CA, USA) and cisplatin (CDDP, *cis*-diaminedichloroplatinum (II); Sigma, MO, USA) were used for apoptosis. For caspase inhibition, z-DEVD-fmk (acetyl Asp-Glu-Val-Asp 7-amido-4-methylcoumarin, Calbiochem), z-IETD-fmk, z-LEHD-fmk, and z-VAD-fml (Transduction Laboratories, KY, USA) were utilized.

2.2. Antibodies (Abs)

Goat anti-eIF3 and rabbit anti-eIF3g Abs were kindly provided by Drs. John W. Hershey (University of California, CA, USA) and Tang K. Tang (Institute of Biomedical Sciences, Taiwan), respectively. In addition, we generated an anti-eIF3g Ab from Balb/c mice that were immunized with the purified recombinant eIF3g-1–229 protein. Mouse anti-GFP and anti-PARP Abs from Santa Cruz Biotechnology (CA, USA), mouse anti-caspases-3 and -7 Abs from Calbiochem, and mouse anti-tubulin and anti-His monoclonal Abs from Sigma were purchased.

2.3. Plasmid construction and site-directed mutagenesis

To generate various pcDNA3His-eIF3g mutants constructs, full-length or deletion fragments of the eIF3g cDNA were inserted into the pcDNA3.1His vectors and GFP-fused eIF3g constructs were generated using the pEGFPC2 vector, as previously reported [15]. Various point mutants of aspartic acid were generated by PCR using site-directed mutant primers.

2.4. In vitro caspase-mediated substrate cleavage assay

^{35}S-methionine (10 μCi, Perkin–Elmer Life Sciences, MA, USA) and pcDNA3-eIF3g constructs were added to the TNT reticulocyte lysate of an in vitro transcription and translation system (Promega, WI, USA). Reticulocytes containing ^{35}S-labeled proteins were reacted with recombinant caspase-3 or -7 (Calbiochem) at 37 °C. After adding SDS-sample buffer and boiling, the lysates were subjected to SDS–PAGE followed by autoradiography.

2.5. Transfection and immunoblotting

T24 cells were seeded in a 6-well plate one day prior to transfection; the cells were then transiently transfected with the

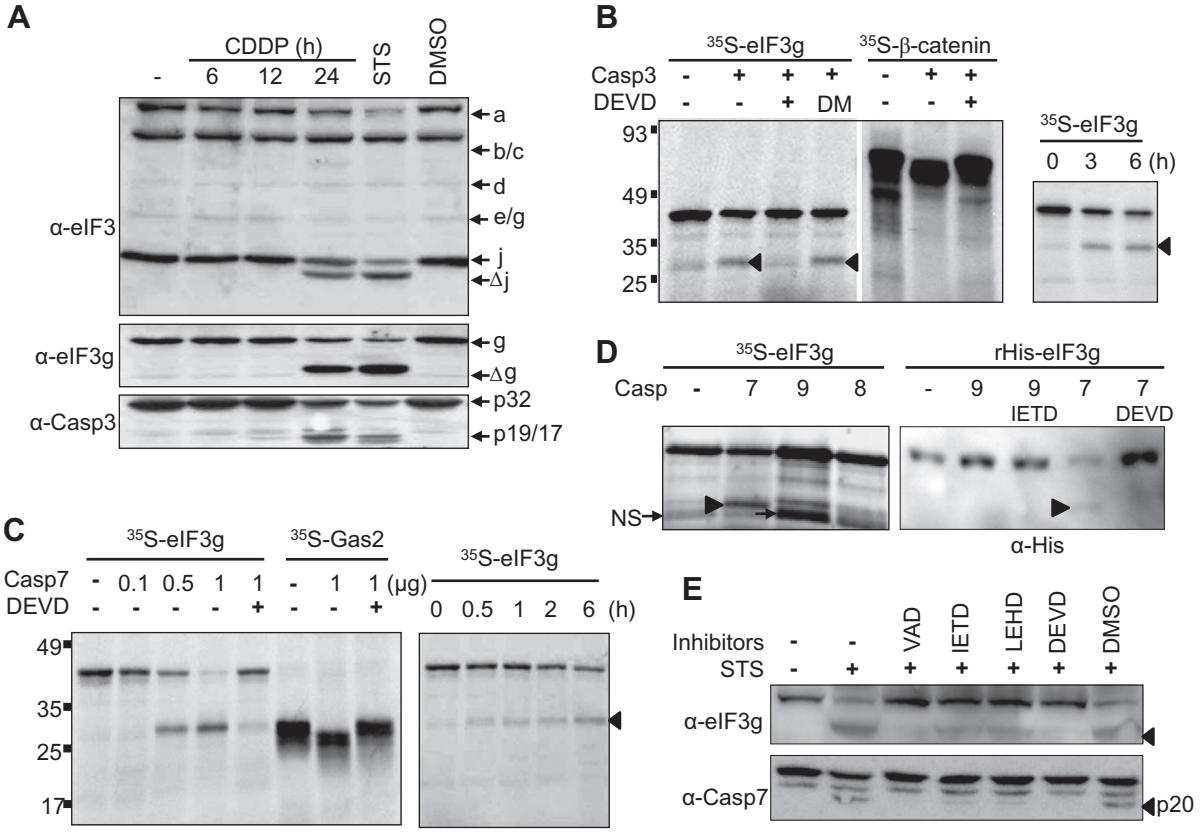

Fig. 1. Cleavage of eIF3g by caspase-3 and -7. (A) T24 cells were treated with either CDDP at 10 μg for 6–12 h or STS at 1 μM for 1 h. Cell lysates were subjected to SDS–PAGE, followed by immunoblotting. Arrows, eIF3 subunits. (B) pcDNA3-eIF3g was in vitro translated, and recombinant caspase-3 (0.3 μg) was reacted with an ^{35}S-eIF3g-containing reticulocyte mixture at 37 °C for 3 h. The reaction mixtures were subjected to SDS–PAGE, followed by autoradiography. β-Catenin was used as a positive substrate for caspase-3. DM, DMSO; arrowhead, cleaved eIF3g. (C) ^{35}S-eIF3g-containing reticulocyte was incubated with recombinant caspase-7 (0.1–1 μg) for 3 h; Gas2 was used as a positive substrate for caspase-7. (D) The ^{35}S-eIF3g-containing reticulocyte or purified recombinant His-eIF3g was incubated with recombinant caspase-7, -8, or -9 (0.5 μg). The reaction mixtures were subjected to SDS–PAGE, followed by autoradiography or immunoblotting. Arrowhead, cleaved eIF3g; arrows, ^{35}S-labeled non-specific bands. (E) T24 cells were treated with several caspase inhibitors (20 μM) and STS (1 μM) for 1 h. Cell lysates were subjected to SDS–PAGE, followed by immunoblotting.

indicated plasmids using the Lipofectamine 2000 reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. For immunoblotting, the cells were lysed with CelLytic M buffer (Sigma) and lysates were subjected to SDS–PAGE and transferred to PVDF membranes. The membranes were blocked with 5% skim milk and then incubated with Abs.

2.6. Fluorescent assay

Cells were transfected with pEGFPC2 for 6 h and then treated with CDDP for 24 h. The cells were harvested, and the lysates were transferred to a 96-well plate. The fluorescent intensity was measured using a fluorometer (FilterMax F3, Molecular Devices, CA, USA) at 488/507 nm. For caspase-3 activity, cells were transfected with pcDNA3His-eIF3g constructs and treated with CDDP the next day. The cells were lysed, and ac-DEVD-amc was added. Fluorescence was measured at 380/450 nm according to the manufacturer's instructions (Cell Signaling, MA, USA).

2.7. Laser-scanning confocal microscopy

T24 cells grown on coverslips were transfected with the pEGFPC2-eIF3g construct. The cells were washed, fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton X-100. After washing, the cells were blocked with 1% BSA and incubated with an anti-karyopherin Ab. The nuclei were stained with DAPI (Sigma), and the cells were visualized using a confocal microscope (LSM510META, Carl Zeiss, Germany).

2.8. Statistics

The quantitative data are presented as the means ± S.D. Statistical significance was assessed by a two-tailed unpaired Student's *t* test. *P*-values <0.05 were considered significant.

3. Results

3.1. eIF3g is cleaved during apoptosis

Previously, we reported that AIF, a mitochondrial apoptotic protein, interacts with eIF3g and inhibits protein synthesis. Interestingly, we found that eIF3g was cleaved when Jurkat or T24 cells were treated with CDDP, a DNA chelating agent. As shown in Fig. 1A, eIF3g and eIF3j were clearly cleaved in T24 cells, when apoptosis induced by STS or CDDP treatment. Thus, we examined which caspases were involved in the eIF3g cleavage during apoptosis. ^{35}S-eIF3g protein was generated using an in vitro translation system and incubated with recombinant caspases. eIF3g was specifically cleaved by caspase-3 and caspase-7 in a time- and dose-dependent manner (Fig. 1B and C); β-catenin and Gas2, substrates of caspase-3 and -7, respectively, were used as positive controls. Furthermore, the specific caspase-3/7 inhibitor, z-DEVD-fmk, blocked eIF3g cleavage, whereas dimethyl sulfoxide (DMSO) did not, indicating that eIF3g might be a novel substrate of caspase-3/7. We then investigated whether ^{35}S-eIF3g or recombinant His-eIF3g was also cleaved by initiator caspases (such as caspase-8 and -9); as shown in Fig. 1D, eIF3g was cleaved by caspase-7 (indicated by an arrowhead), but not by caspase-8 or -9. To verify

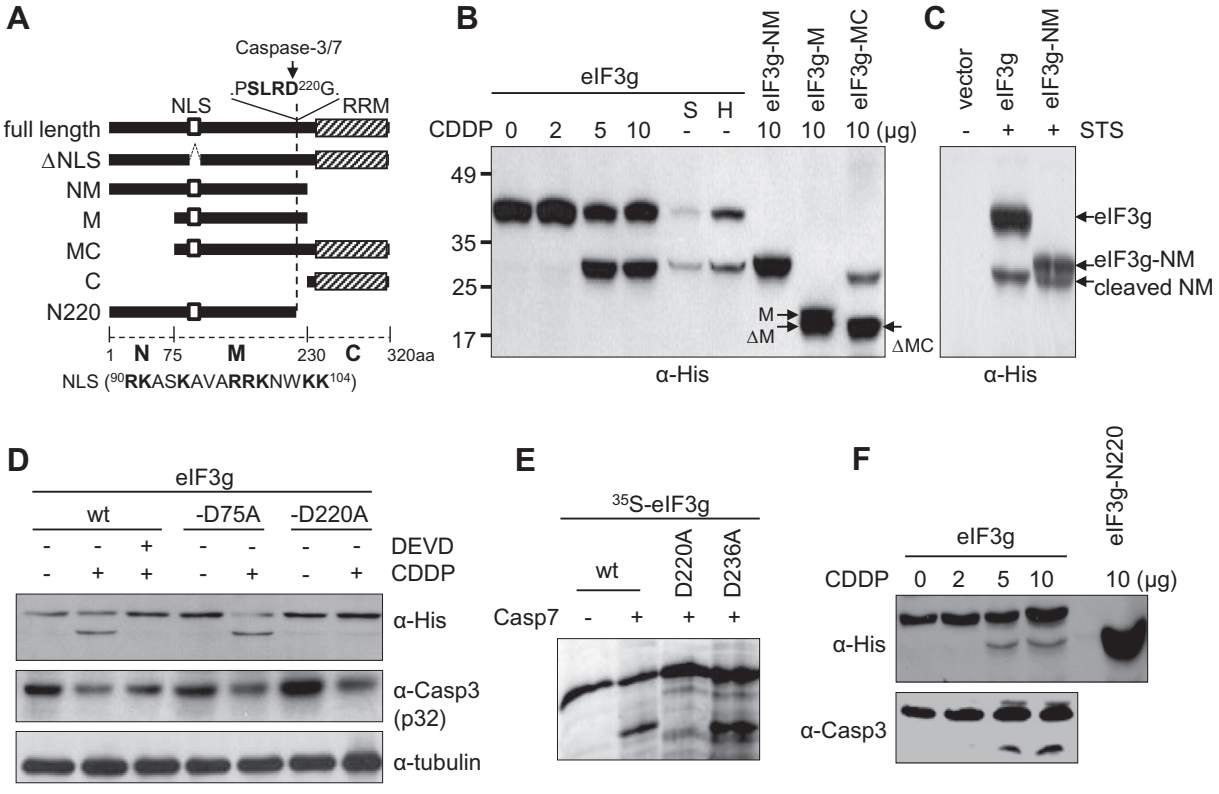

Fig. 2. eIF3g is cleaved at the Asp^{220} by caspases. (A) Scheme of the eIF3g deletion fragments. N, N-terminus; M, middle; C, C-terminus; NLS, nuclear localization sequence; RRM, RNA recognition motif. (B) T24 cells were transfected with pcDNA3His-eIF3g or deletion mutants. One day later, the cells were treated with CDDP (μg) for 24 h. Cell lysates were subjected to SDS–PAGE, followed by immunoblotting. S, STS (1 μM, 4 h); H, H_2O_2 (1 mM, 6 h). (C) Cells were transfected with plasmids and treated with STS (1 μM, 4 h). (D) pcDNA3His-eIF3g or site-directed mutants were transfected and treated with CDDP. Decreased expression of the proform (p32) of caspase-3 was detected in the cell lysates treated with CDDP. (E) pcDNA3His-eIF3g or site-directed mutants were in vitro translated and incubated with recombinant caspase-7 for 3 h. (F) Cells were transfected with pcDNA3His-eIF3g or eIF3g-N220. One day later, cells were treated with CDDP for 24 h, and the lysates were analyzed by immunoblotting.

eIF3g cleavage, several caspase-inhibitors, such as z-DEVD-fmk (caspase-3/7), z-VAD-fmk (pan-caspases), z-LEHD-fmk (caspase-8), and z-IETD-fmk (caspase-9) were added prior to STS treatment. We observed that eIF3g cleavage and caspase-7 activation were blocked in the z-VAD and z-DEVD-treated cells, but were only partially blocked in the z-LEHD and z-IETD-treated cells, indicating that eIF3g is not a substrate of initiator caspases, such as caspase-8 and -9 (Fig. 1E).

3.2. eIF3g is cleaved at Asp^{220} by caspases

eIF3g consists of 320 amino acids (aa) and has a molecular weight of 42–44 kDa. To delineate the cleavage site and the functional domain, eIF3g was divided into N-terminal (N), middle (M), and C-terminal (C) domains (Fig. 2A). When T24 cells were transfected with pcDNA3His-eIF3g or its deletion mutants, eIF3g and the variants, including -NM (1–229 aa), -M (75–229 aa), and -MC (75–320 aa) were notably cleaved by CDDP treatment, with cleavage of eIF3g-M and eIF3g-MC generating N-terminal products of similar sizes (Fig. 2B). Furthermore, the cleavage products of eIF3g and eIF3g-NM were similar in size with STS treatment (Fig. 2C). These findings indicate that the cleavage site of eIF3g is in the C-terminal region of the eIF3g-M fragment. Among several XXXD sites, the SLRD^{220}G motif represents an SXXD site that is reported to be a recognition site for caspases [16,17]. We generated an eIF3g-D220A mutant in which the Asp (D) residue is substituted with Ala (A). When cells were transfected with pcDNA3His-eIF3g-D220A and treated with CDDP, cleavage products were detected for wild-type eIF3g and the eIF3g-D73/75A mutant (substitution of Asp^{73} and Asp^{75}) but not for the eIF3g-D220A mutant (Fig. 2D), a result that was verified using recombinant caspase-7 and ^{35}S-eIF3g-D220A (Fig. 2E). Overexpression of the eIF3g-N220 (1–220 aa) construct after transfection yielded similarly sized eIF3g cleavage products (Fig. 2F), indicating that eIF3g is cleaved at SLRD^{220} during apoptosis.

3.3. Nuclear translocation of the eIF3g N-terminus

Interestingly, we found that cytosolic eIF3g can translocate to the nucleus during apoptosis. Cells were transfected with pEGFPC2-eIF3g or the mutants and treated with CDDP, and the cells were analyzed using confocal microscopy. In the GFP-eIF3g-cells, cytosolic eIF3g was translocated to the nucleus when the cells were treated with CDDP (Fig. 3A). However, cytosol-localized GFP-eIF3g-D220A and nuclear-localized GFP-eIF3g-N220 remained unchanged. To examine the cellular distribution of the eIF3g fragments, several GFP-fused deletion mutants were generated, and their expression was evaluated by immunoblotting (Fig. 3B). Confocal microscopy showed the nuclear accumulation of the M-region containing eIF3g-NM, -M, and -MC (Fig. 3C). This nuclear translocation of eIF3g-M indicates that the M-region may contain the nuclear localization sequence (NLS). To identify the predicted NLS, two suspected regions were mutated: deletion of the basic amino acid-rich region [Δ90–104 (ΔNLS)] and mutation of the PY residue at 170–171 aa (PYmut). After eIF3g-ΔNLS and -PYmut were assessed by immunoblotting (Fig. 3D), their cellular

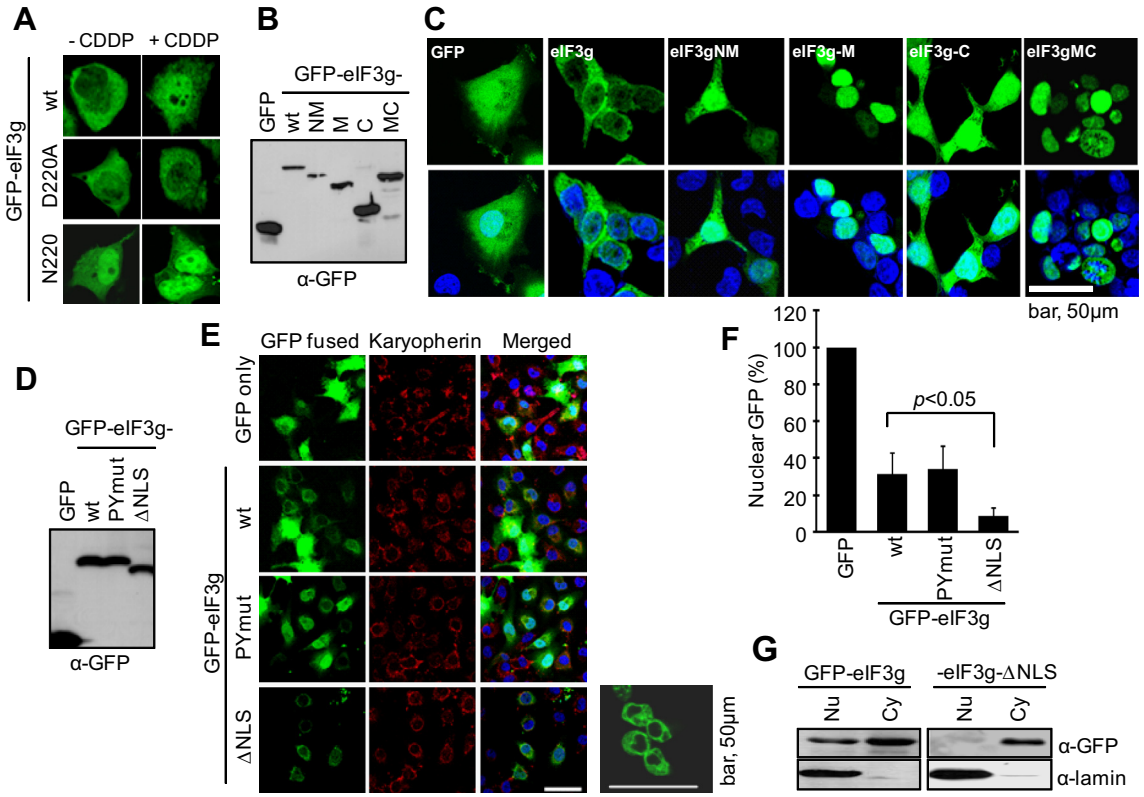

Fig. 3. Nuclear translocation of the N-terminal fragment of eIF3g. (A) T24 cells grown on cover slips were transfected with pEGFPC2-eIF3g, -D220A, or -N220 and treated with CDDP. The cells were fixed and analyzed using confocal microscopy. (B) Cells were transfected with pEGFPC2-eIF3g or deletion fragments for 1 day, and the cell lysates were analyzed by immunoblotting. (C) Cells grown on cover slips were transfected with pEGFPC2-eIF3g variants and analyzed using confocal microscopy. Blue, DAPI for nuclear staining. (D) Cells were transfected with pEGFPC2-eIF3g, -ΔNLS, or -PYmut for 1 day and then analyzed by immunoblotting. (E) Cells grown on cover slips were transfected with pEGFPC2-eIF3g variants, fixed, stained, and analyzed using confocal microscopy. Karyopherin was used as a nuclear pore protein. (F) The nuclear accumulation of GFP was evaluated. Cells in an area of 100 × 100 μm were scored. The mean ± S.D. values from three independent experiments performed in triplicate are shown. (G) Cells were transfected with pEGFPC2-eIF3g or -ΔNLS; the nuclear and cytosolic fractions were isolated and subjected to SDS–PAGE, followed by immunoblotting. Lamin was used as a positive control for the nuclear fraction.

distributions were analyzed using confocal microscopy (Fig. 3E). GFP-eIF3g-ΔNLS was distributed in the cytosol, whereas eIF3g and eIF3g-PYmut were located in both the cytosol and nucleus. As shown in Fig. 3F, the cells were scored as "nuclear" if the nucleus exhibited a greater fluorescence intensity than the cytoplasm, and indeed the deletion of the putative NLS decreased the translocation of eIF3g to the nucleus by one-third. We then examined the distributions of GFP-eIF3g and -ΔNLS in the nucleus versus the cytosol, and detected eIF3g-ΔNLS in the cytosolic fraction (Fig. 3F).

3.4. The N-terminus of eIF3g indirectly activates caspases

Alterations in protein synthesis after CDDP treatment were investigated. Newly synthesized proteins were found to be strongly decreased, and eIF3g cleavage and caspase-3 activation were observed, depending on the CDDP treatment (data not shown). When cells were cotransfected with eIF3g-siRNA and GFP plasmid, GFP protein synthesis was decreased with eIF3g knockdown (Fig. 4A). In addition, the cells treated with eIF3g-siRNA showed cell growth retardation (Fig. 4B), indicating that eIF3g is important for the translation system. To determine the role of the eIF3g fragments, cells were transfected with eIF3g, -ΔNLS, -M, -N220, or -C and treated with CDDP; cell lysates were reacted with ac-DEVD-amc, a fluorogenic substrate for caspase-3, and fluorescence was measured (Fig. 4C). Interestingly, NLS-containing eIF3g-M and -N220 showed increased caspase-3 activity, even in the cells that were not treated with CDDP, whereas the caspase-3 activity in cells transfected with the vector control, eIF3g wt, -C, or -ΔNLS was not significantly different. Furthermore, caspase-3 and -7 were activated when the cells were transfected with eIF3g-N220 (Fig. 4D) or eIF3g-NM (data not shown), indicating that caspase-mediated eIF3g cleavage may participate in apoptotic process. However, the recombinant eIF3g-N220 did not directly activate recombinant caspase-3 or caspase-7 (data not shown). These data suggest that the NLS-containing N-terminal region of eIF3g may play a role in the nucleus during apoptosis.

3.5. DNase activity of the eIF3g N-terminus

Because it had been reported that eIF3g is able to bind to AIF, which translocates to the nucleus and induces large-scale DNA fragmentation [15], we investigated whether eIF3g has DNase activity. To test this possibility, eIF3g protein was in vitro translated, and ^{35}S-labeled eIF3g was reacted with genomic DNA (gDNA). As shown in Fig. 5A, gDNA was remarkably degraded in the ^{35}S-eIF3g-reticulocyte assay in the presence of caspase-7, but remained intact in the absence of caspase-7 or in the additional presence of z-DEVD-fmk. To verify the DNase activity of cleaved eIF3g, *Escherichia coli BL21* (*DE3*) cells were transformed with pET28a-eIF3g, -NM, or -N220, and protein expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG). Lysates of cells were incubated with gDNA prior to protein purification, and gDNA was significantly degraded in the lysates of the eIF3g-NM- and -N220-expressing cells (Fig. 5B). The recombinant proteins were also purified and reacted directly with gDNA, which was rapidly degraded in the presence of eIF3g-NM but not eIF3g (Fig. 5C). The degradation of gDNA by eIF3g-NM and -N220 occurred in a

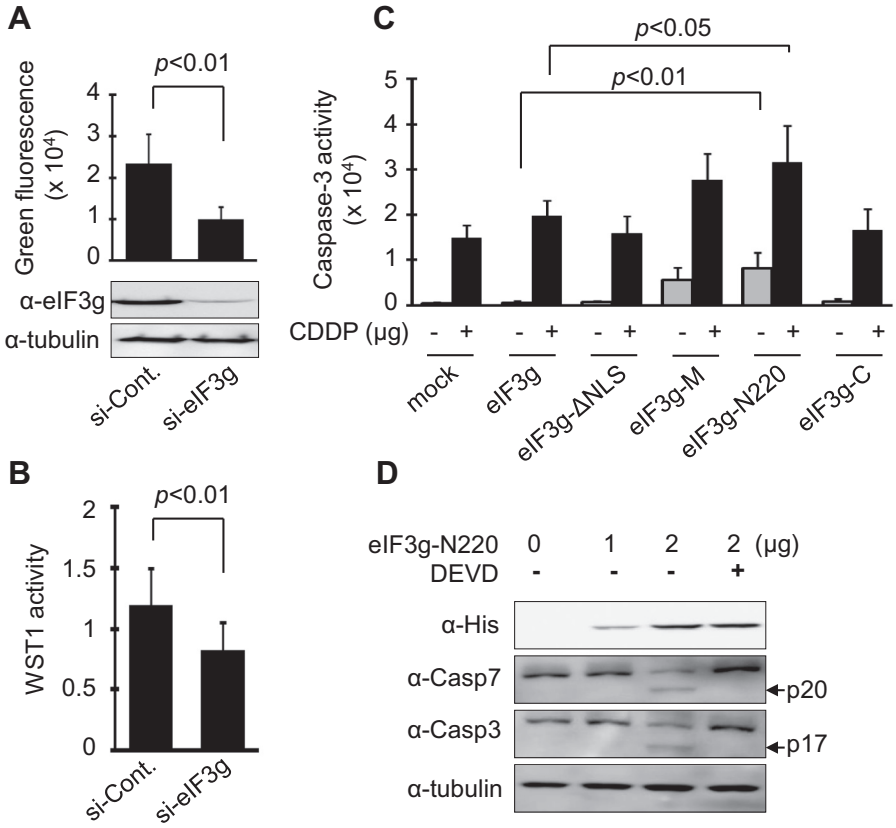

Fig. 4. The N-terminal region of eIF3g indirectly activates caspases. (A) Cells were cotransfected with peGFPC2 vector and eIF3g-siRNA (50 nM) for 2 days. GFP fluorescence was measured using a fluorometer, and eIF3g expression was examined by immunoblotting. The mean ± S.D. values from three independent experiments performed in triplicate are shown. (B) Cells were transfected with eIF3g-siRNA and analyzed at 3 days after cell proliferation using the WST1 reagent. (C) Cells were transfected with pcDNA3His-eIF3g or several fragments and treated with CDDP (10 μg, 24 h). Cells were lysed and reacted with ac-DEVD-amc, and the lysates were measured using a fluorometer. (E) Cells were transfected with pcDNA3His-eIF3g-N220, and the cell lysates were analyzed by immunoblotting after 2 days.

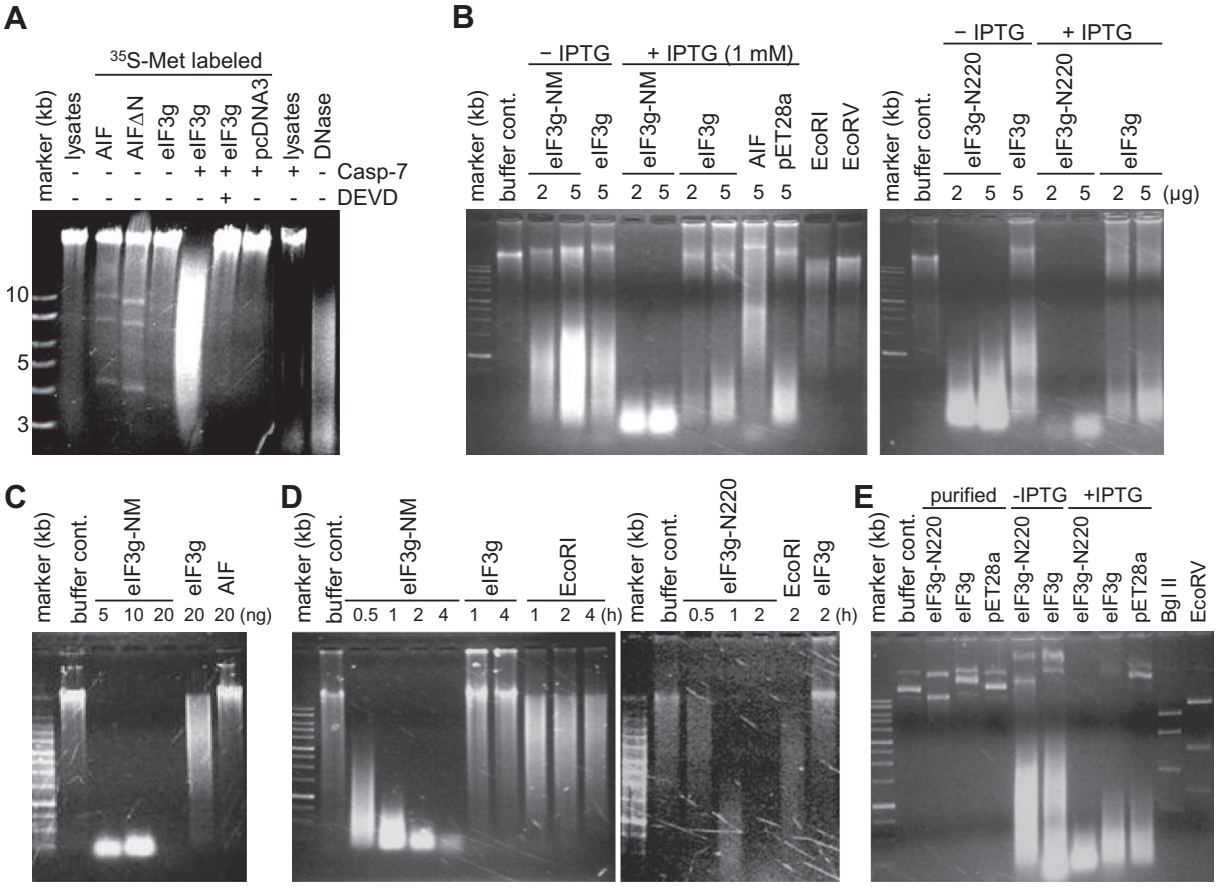

Fig. 5. DNase activity of the N-terminal region of eIF3g. (A) In vitro-translated eIF3g was incubated with recombinant caspase-7 and reacted with gDNA; the samples were then electrophoresed and stained with ethidium bromide. (B) *E. coli BL21* (*DE3*) cells were transformed with pET28a-eIF3g, -NM, or -N220, and IPTG was added for protein induction. The cells were lysed, and 2–5 μg of protein was incubated with gDNA for 1 h. The nucleases EcoR I and EcoR V were used as positive controls. (C and D) The recombinant eIF3g wt, -NM, and -N220 proteins were purified and directly reacted with gDNA for 4 h (C), or 5 μg of protein was reacted for different time intervals (D). (E) pEG202LexA (10.2 kb) plasmids were directly reacted with the purified eIF3g wt or -N220. *E. coli* lysates were also used (−/+ IPTG). Nucleases Bgl II and EcoR V were used as positive controls.

time-dependent manner, and the observed DNase activity was stronger than that observed for EcoR I DNase (Fig. 5D). Lastly, when plasmid pEG202LexA (10.2 kb) was incubated with the purified eIF3g-N220, the plasmid was not digested but just cut one time (Fig. 5E). Together, although the exact mechanism remains unclear, our results suggest that the NLS-containing N-terminus of eIF3g possesses strong DNase activity.

4. Discussion

Caspases are prominent regulators in apoptosis and play roles in initiating and executing the caspase cascade to dismantle the cellular survival system, including the process of translation for protein synthesis. During the execution phase of apoptosis, downstream effector caspases cleave a variety of substrate proteins that are known to play important roles in cellular homeostasis, the organization and maintenance of the cellular structure, and the apoptotic process. Caspase-mediated proteolytic cleavage of several translation factors typically yields characteristic cleaved products. eIF3j is normally involved in bridging the 40S ribosomal subunit and eIF3 [18], but cleaved eIF3j has an altered affinity for the eIF3–40S complex [13]. Indeed, the affinity of eIF3j for the 40S subunit is significantly reduced by the caspase-3-mediated removal of 16 residues at the C-terminus, and the resulting eIF3–40S complex is less stable and less efficient in translation [19]. As we demonstrated in this study, eIF3g was cleaved by caspase-3 and -7 at a site near the RNA recognition motif (RRM) domain, resulting in decreased protein synthesis. Although we could not detect the RRM-containing C-terminal fragment of eIF3g, the large N-terminus of eIF3g was clearly detected by autoradiography and immunoblotting. Cleavage of eIF3g by executioner caspases is predicted to significantly contribute to the collapse of the translation initiation machinery.

In general, protein synthesis is significantly down-regulated during apoptosis, and we found that cell growth was suppressed and protein production significantly decreased when T24 cells were transfected with an siRNA targeting eIF3g (Fig. 4). In addition, the eIF3g protein was constitutively expresssed in several cell lines, including colon and gastric tumor cells (data not shown). Interestingly, the nuclear-localized eIF3g N-terminus (-NM, -M, and -N220) induced caspase activation and DNA degradation, thus exhibiting apoptotic characteristics (Figs. 4 and 5). However, Machuy et al. [20] used RNAi screening for apoptosis regulators and observed the inhibition of apoptosis via RNAi-induced eIF3g suppression. Although they showed a contrasting and intriguing result for eIF3g, eIF3g is clearly one of the core subunits of the eIF3 complex [4,8,21], which is essential for translation initiation and cell growth; indeed, these core subunits are conserved in yeast and mammals. We are currently planning experiments to investigate the molecular mechanisms by which eIF3g-N220 but not eIF3g is able to activate caspases and degrade DNA, and we are focusing on determining potential binding partners in the nucleus.

In conclusion, our study reveals a novel role of the eIF3g N-terminus, the executioner caspase-mediated cleavage product, in the translation initiation machinery and in DNA degradation.

Acknowledgments

We are grateful to Dr. Tang K. Tang (Institute of Biomedical Sciences, Taiwan) and Dr. John W. Hershey (University of California, Davis, CA) for the generous gift of the anti-eIF3g and anti-eIF3 Abs, respectively. This work was supported by the Creative Allied Project (CAP) grant funded by the Korea Research Council of Fundamental Science and Technology (KRCF) and by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIP) (R11-2011-0001386).

References

[1] Hannig, E.M. (1995) Protein synthesis in eukaryotic organisms: new insights into the function of translation initiation factor eIF-3. Bioessays 17, 915–919.
[2] Hinnebusch, A.G. (2006) EIF3: a versatile scaffold for translation initiation complexes. Trends Biochem. Sci. 31, 553–562.
[3] Hershey, J.W., Asano, K., Naranda, T., Vornlocher, H.P., Hanachi, P. and Merrick, W.C. (1996) Conservation and diversity in the structure of translation initiation factor EIF3 from humans and yeast. Biochimie 78, 903–907.
[4] Cuchalova, L., Kouba, T., Herrmannova, A., Danyi, I., Chiu, W.L. and Valasek, L. (2010) The RNA recognition motif of eukaryotic translation initiation factor 3g (eIF3g) is required for resumption of scanning of posttermination ribosomes for reinitiation on GCN4 and together with eIF3i stimulates linear scanning. Mol. Cell. Biol. 30, 4671–4686.
[5] Browning, K.S., Gallie, D.R., Hershey, J.W., Hinnebusch, A.G., Maitra, U., Merrick, W.C. and Norbury, C. (2001) Unified nomenclature for the subunits of eukaryotic initiation factor 3. Trends Biochem. Sci. 26, 284.
[6] Mayeur, G.L., Fraser, C.S., Peiretti, F., Block, K.L. and Hershey, J.W. (2003) Characterization of eIF3k: a newly discovered subunit of mammalian translation initiation factor eIF3. Eur. J. Biochem. 270, 4133–4139.
[7] Zhou, C., Arslan, F., Wee, S., Krishnan, S., Ivanov, A.R., Oliva, A., Leatherwood, J. and Wolf, D.A. (2005) PCI proteins eIF3e and eIF3m define distinct translation initiation factor 3 complexes. BMC Biol. 3, 14.
[8] Phan, L., Zhang, X., Asano, K., Anderson, J., Vornlocher, H.P., Greenberg, J.R., Qin, J. and Hinnebusch, A.G. (1998) Identification of a translation initiation factor 3 (eIF3) core complex, conserved in yeast and mammals, that interacts with eIF5. Mol. Cell. Biol. 18, 4935–4946.
[9] Clemens, M.J., Bushell, M., Jeffrey, I.W., Pain, V.M. and Morley, S.J. (2000) Translation initiation factor modifications and the regulation of protein synthesis in apoptotic cells. Cell Death Differ. 7, 603–615.
[10] Bushell, M., Poncet, D., Marissen, W.E., Flotow, H., Lloyd, R.E., Clemens, M.J. and Morley, S.J. (2000) Cleavage of polypeptide chain initiation factor eIF4GI during apoptosis in lymphoma cells: characterisation of an internal fragment generated by caspase-3-mediated cleavage. Cell Death Differ. 7, 628–636.
[11] Marissen, W.E. and Lloyd, R.E. (1998) Eukaryotic translation initiation factor 4G is targeted for proteolytic cleavage by caspase 3 during inhibition of translation in apoptotic cells. Mol. Cell. Biol. 18, 7565–7574.
[12] Gradi, A., Svitkin, Y.V., Imataka, H. and Sonenberg, N. (1998) Proteolysis of human eukaryotic translation initiation factor eIF4GII, but not eIF4GI, coincides with the shutoff of host protein synthesis after poliovirus infection. Proc. Natl. Acad. Sci. USA 95, 11089–11094.
[13] Bushell, M., Wood, W., Clemens, M.J. and Morley, S.J. (2000) Changes in integrity and association of eukaryotic protein synthesis initiation factors during apoptosis. Eur. J. Biochem. 267, 1083–1091.
[14] Satoh, S., Hijikata, M., Handa, H. and Shimotohno, K. (1999) Caspase-mediated cleavage of eukaryotic translation initiation factor subunit 2alpha. Biochem. J. 342 (Pt 1), 65–70.
[15] Kim, J.T. et al. (2006) Apoptosis-inducing factor (AIF) inhibits protein synthesis by interacting with the eukaryotic translation initiation factor 3 subunit p44 (eIF3g). FEBS Lett. 580, 6375–6383.
[16] Fischer, U., Janicke, R.U. and Schulze-Osthoff, K. (2003) Many cuts to ruin: a comprehensive update of caspase substrates. Cell Death Differ. 10, 76–100.
[17] Earnshaw, W.C., Martins, L.M. and Kaufmann, S.H. (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu. Rev. Biochem. 68, 383–424.
[18] ElAntak, L., Tzakos, A.G., Locker, N. and Lukavsky, P.J. (2007) Structure of eIF3b RNA recognition motif and its interaction with eIF3j: structural insights into the recruitment of eIF3b to the 40S ribosomal subunit. J. Biol. Chem. 282, 8165–8174.
[19] Fraser, C.S., Lee, J.Y., Mayeur, G.L., Bushell, M., Doudna, J.A. and Hershey, J.W. (2004) The j-subunit of human translation initiation factor eIF3 is required for the stable binding of eIF3 and its subcomplexes to 40S ribosomal subunits in vitro. J. Biol. Chem. 279, 8946–8956.
[20] Machuy, N., Thiede, B., Rajalingam, K., Dimmler, C., Thieck, O., Meyer, T.F. and Rudel, T. (2005) A global approach combining proteome analysis and phenotypic screening with RNA interference yields novel apoptosis regulators. Mol. Cell. Proteomics 4, 44–55.
[21] Herrmannova, A. et al. (2012) Structural analysis of an eIF3 subcomplex reveals conserved interactions required for a stable and proper translation pre-initiation complex assembly. Nucleic Acids Res. 40, 2294–2311.